REMARKS

Amendment of the specification and reconsideration of the Official communication from the Examiner of December 12, 2003 is respectfully requested by Applicants.

The specification has been amended to comply with the Examiner's comment that the specification contains sequences that are not identified by the requisite SEQ ID NO's.

In response, Applicants have amended their specification to insert SEQ ID NO's after each occurrence of a sequence.

Applicants submit that their application is now in condition for allowance, and favorable reconsideration of their application in light of the above amendments and is respectfully requested. Allowance of claims 18-25 at an early date is earnestly solicited.

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The Examiner is hereby authorized to charge any fees associated with this Amendment to Deposit Account No. 50-0877 02-2958. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

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translational modifications or interactions with other factors without requiring the presence of protein-specific antibodies.

An advantage of viral epitopes compared to cellular epitopes is that these protein sequences do not usually occur in bacterial and eukaryotic proteins and thus no cross-reactions would be expected in bacterial or cellular systems.

A viral epitope frequently described in the literature that is used for such analyses is derived from the haemagglutinin of the human influenza virus. This epitope has the amino acid sequence YPYDVPDYA (98 - 106) (SEQ ID NO: 1) (Field, J. et al. (1988), Mol. Cell. Biol. Vol. 8, No. 5, 2159-2165 and Wilson et al., Cell 37, 767 - 778, (1984)). Monoclonal antibodies (mAB) against this epitope have been described and are available such as for example the mAB 12CA5 (P.A. Kolodziej and Young, R.A., Meth. Enzymol. (1991), Vol. 194, 508-519; Chen, Y.-T. et al. (1993), Proc. Natl. Acad. Sci., Vol. 90, 6508-6512)) and the anti-HA-BabCo.

However, a disadvantage of these antibodies is that their affinity is not high enough and therefore the epitope-specific antibodies have to be used in a high concentration for a sensitive detection of the corresponding fusion proteins which can lead to unspecific interactions which for example become apparent as cross-reactions in a Western blot (cf. Chen et al., p. 6510).

The inadequate affinity also leads to a lower sensitivity of the known anti-HA mAB.

Therefore the object of the invention was to provide monoclonal antibodies against the viral epitope YPYDVPDYA (SEQ ID NO: 1) which have a higher affinity and which are thus suitable for highly sensitive haemagglutinin tests or HA fusion protein tests and give reproducible results.

According to the invention monoclonal antibodies are provided which recognize the epitope having the amino acid sequence YPYDVPDYA (98 - 106) (SEQ ID NO: 1) of the haemagglutinin of the human influenza virus as well as corresponding fragments thereof and have an affinity of $> 10^8$ M^{-1}, in particular of 10^9 to 10^{10} M^{-1}. In this connection

epitope fragments are understood in particular as those amino acid sequences which correspond to at least 70 % of the sequence YPYDVPDYA (SEQ ID NO: 1) or are shortened by at least one to two terminal amino acids.

In order to produce the monoclonal antibodies, small mammals, preferably rats, such as e.g. Lou/C rats or mice such as e.g. BalbC mice or rabbits are immunized with a HA peptide synthesized by standard methods. An uncoupled HA peptide or a HA peptide which is optionally coupled N-terminally or C-terminally to a carrier protein or a HA fusion protein is used as the antigen. Keyhole limpet haemocyanin (KLH) or bovine serum albumin (BSA) were preferably used as carrier proteins. Subsequently B lymphocytes were isolated from the spleen of the animals and immortalized by cell fusion with suitable myeloma cells or by other known methods such as e.g. by means of oncogenes (Jonak, Z.L. et.al., (1988) Adv. Drug Rev. 2:207-228) or in an electrical field (Zimmermann, U. (1982), Biochim. Biophys. Acta 694:227-277). The cell fusion was preferably carried out according to the invention with spleen cells of Lou/C rats and myeloma cells from the mouse line P3x63-Ag8,653 (Kearney, J.F. et al (1979), J. Immunol. 123, 1548-1550).

In this process the lymphocytes and the myeloma cells are fused by known methods, in particular by polyethylene glycol fusion (PEG), virus fusion or electrofusion and the hybrid cells (cell clones) that are formed are also selected by known methods such as e.g. by using selection media.

Thus for example positive clones were firstly tested with HA peptides and then with HA fusion proteins. In a first screen a biotinylated HA peptide e.g: Bio-C-HA (acetyl-**YPYDVPDYA**GSGSK (ε-biotinoyl)-amide) (SEQ ID NO: 2) or Bio-N-HA (biotinoyl-ε-Aca-SGSG**YPYDVPDYA**-amide) (SEQ ID NO: 3) was used and a HA-tagged glutathione-S-transferase (GST) was used in a second screen. Clones that were again positive were subsequently examined with regard to their affinity with the aid of plasmon resonance in a BIACORE® (registered trademark of Biacore AB) system and they were selected.

The hybrid cells were cloned, cultured and multiplied according to known methods and optionally stored in liquid nitrogen.

The cell lines R 3F10, R 3A12 and R 6D12 were established as the most active cell clones with a stable antibody production. The hybridoma R 3A12 was deposited on the 08.10.1996 at the "Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ)", Mascheroder Weg 1b, 38124 Braunschweig under the number DSM ACC2286.

For the antibody isolation the hybrid cells were further propagated in cell culture or optionally in vivo by transplantation as ascites tumours. The mABs were isolated from the cell culture supernatants or optionally from the ascites fluid of the tumour-carrying experimental animals.

The mAB produced in high concentration by the hybrid cells which are characterized by an excellent specificity and binding strength for the YPYDVPDYA (SEQ ID NO: 1) epitope of the haemagglutinin of the human influenza virus or for corresponding epitope fragments are obtained according to the invention. They enable the highly sensitive detection and isolation of haemagglutinin as well as of proteins to which the HA epitope YPYDVPDYA (SEQ ID NO: 1) has been attached.

The affinity of the mAB according to the invention is $> 10^8\ M^{-1}$. Thus the affinity of the mAB 3F10 at $10^{10}\ M^{-1}$ is approximately 30-fold higher than that of the known antibodies 12CA5 ($10^8\ M^{-1}$) and BabCo ($10^7\ M^{-1}$). The affinity of the mABs 3A12 and 6D12 according to the invention is $10^9\ M^{-1}$ and is thus also higher than that of the known antibodies. The mABs according to the invention can be used in much lower concentrations and cross-reactions can be almost completely ruled out. They enable an improved sensitivity of the detection. It has turned out that they recognize native HA of the influenza virus, modified HA as well as HA fusion proteins. Hence they can be used very well for the determination of proteins in known detection reactions such as e.g. a solid phase two-side binding test.

Figure legends

Figure 1:
Affinities of the mABs according to the invention compared to the mAB 12CA5 and anti-HA BabCo.

Figure 2:
Immunoblot analysis of a HA-modified glutathione-S-transferase protein with a mAB (clone 3F10) according to the invention and anti-HA of the prior art (clone 12CA5); a) detection with anti-rat-peroxidase, b) anti-rat biotin/streptavidin-peroxidase.

Figure 3:
Immunoblot analysis of a HA-modified glutathione-S-transferase protein with a mAB (clone 3F10) according to the invention and anti-HA of the prior art (clone 12CA5).

Figure 4:
Immunoblot analysis of a HA-modified glutathione-S-transferase protein with enzyme (peroxidase) conjugates of an antibody according to the invention (clone 3F10) and an antibody of the prior art (clone 12CA5).

Figure 5:
Immunoprecipitation of a HA-modified green fluorescent (GFP-HA) protein with a mAB (clone 3F10) according to the invention and anti-HA of the prior art (clone 12CA5).

Subsequently the invention is further elucidated by the following examples of use.

Example 1:

Production of clones R 3F10, R 3A12 and R 6D12

HA peptide preparation

The following peptides were synthesized:
Bio-C-HA (acetyl-**YPYDVPDYA**GSGSK (ε-biotinoyl)-amide) (SEQ ID NO: 2)
Bio-N-HA (biotinoyl-ε-Aca-SGSG**YPYDVPDYA**-amide) (SEQ ID NO: 3)
KLH-MPS-CUZU-HA-C
KLH-MPS-CUSU-HA-N